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Lipid Composition of Plasma Membranes and Endomembranes Prepared from Roots of Barley (*Hordeum vulgare* L.)[1]

Effects of Salt

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ABSTRACT

Membrane fractions enriched in endoplasmic reticulum (ER), tonoplast and Golgi membranes (TG) and plasma membranes (PM) were prepared from barley (*Hordeum vulgare* L. cv CM 72) roots and the lipid compositions of the three fractions were analyzed and compared. Plants were grown in an aerated nutrient solution with or without 100 millimolar NaCl. Each membrane fraction had a characteristic lipid composition. The mole per cent of the individual phospholipids, glycolipids, and sterols in each fraction was not altered when roots were grown in 100 millimolar NaCl. The ER had the highest percentages of phosphatidylinositol and phosphatidylcholine of the three fractions (7 and 45 mole per cent, respectively, of the total lipid). The TG contained the highest percentage of glycosylceramide (13 mole per cent). The PM had the highest percentage of phosphatidylserine (3 mole per cent) and nearly equal percentages of phosphatidylethanolamine (15 mole per cent and phosphatidylcholine (18 mole per cent). The most abundant sterols in membranes prepared from barley roots were stigmasterol (10 mole per cent), sitosterol (50 mole per cent), and 24ζ-methylcholesterol (40 mole per cent of the total sterol). Salt-treated plants contained a slightly higher percentage of stigmasterol than controls. The percentage of stigmasterol increased with age and a simple cause and effect relationship between salt treatment and sterol composition was not observed.

Membrane lipids form a physical barrier to the movement of the water soluble components of cells. They also provide a matrix for membrane transport proteins. At the PM,[3] the combination of the lipid barrier and the selective ion transporters allows cells to accumulate essential ions while excluding ions which are toxic. The exclusion of Na^+, for example, is believed to be an important trait of salt-tolerant barley cultivars (21). Selective transport also occurs at the tonoplast and other endomembranes. The selectivity of each membrane varies with the types of ion channels and pumps that are present, while the efficacy of each membrane as a barrier may vary with the type and proportion of its lipid components (4, 5, 7). The major lipid components of plant membranes are phospholipids. glycolipids, and sterols. The specific proportions of these lipids in the PM (24, 29, 30) and the tonoplast (25, 32) are known for a variety of plant tissues. Only one study (34) has examined the lipid composition of both the PM and tonoplast prepared from a single source.

There is some evidence that salt stress can induce changes in plant membrane lipids. A survey of plant species of varying salt-tolerance reported an increase in the ratio of glycolipid to phospholipid in the roots of both a halophyte, *Atriplex gmelinia*, and the salt-sensitive cucumber, *Cucumis sativa* L., when plants were grown in increasing concentrations of NaCl (18). In *Citrus* roots, the sterol composition was altered in salt-stressed plants and some of these changes occurred at the PM (8, 9). It was not known if salt affects the lipid composition of the PM or endomembranes of barley.

Membrane fractions that are enriched in PM, tonoplast, and ER are prepared by centrifuging a microsomal pellet prepared from barley roots through a sucrose step gradient (11, 12). When barley plants are grown in 100 mM NaCl the distribution of marker enzymes on sucrose gradients does not change, but the protein compositions of the PM, endomembrane, and cytoplasmic fractions are altered (19, 20), and a Na^+/H^+ exchange is activated in the tonoplast membranes. Shoot growth is reduced but the plants show no signs of injury (19). In this study we identify and quantify the phospholipids, glycolipids, and sterols contained in the three membrane fractions. The effect of a high but noninjurious concentration

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[3] Abbreviations: PM, plasma membrane; 2D, two-dimensional; CI-MS, chemical ionization mass spectrometry; DPG, diphosphatidylglycerol (cardiolipin); LSIMS, liquid secondary ion mass spectrometry; lysoPC, lysophosphatidylcholine; lysoPE, lysophosphatidylethanolamine; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TG, tonoplast and Golgi membranes; 24ζ-methylcholesterol, campesterol or dihydrobrassicasterol, orientation of methyl group at carbon 24 not determined.

of salt, 100 mM NaCl, on the lipid composition of each fraction is also reported.

MATERIALS AND METHODS

Plant Materials

Seeds of barley (*Hordeum vulgare* L. cv CM 72) were sown above aerated nutrient solutions (19). Control plants were grown above a full nutrient solution (13), and salt-grown plants were grown above a full nutrient solution plus 100 mM NaCl. Solutions were adjusted daily to pH 5.6 with $Ca(OH)_2$. Seed germination and root growth was reduced by salt. In order to obtain 60 g fresh weight of d 7 roots per treatment, three containers of control plants and four containers of salt-treated plants were required (400 seeds per container).

Membrane Preparation

Roots were homogenized and membranes were fractionated by differential centrifugation and sucrose step gradients as described (12). Membrane fractions (6 mL) were collected from the sample/22%, 22/30%, and 34/40% interfaces of the sucrose step gradients. The fractions were washed with a buffered solution of 150 mM KCl and membranes were pelleted in a Beckman[4] 42.1 rotor at 100,000*g*. The membrane pellets were resuspended in 2 mL of buffer consisting of 0.25 M sucrose and 2 mM DTT in 5 mM Pipes-KOH (pH 7.2), and stored frozen at −70°C. The identity and purity of the three fractions, as defined by enzyme markers and immunoblots of proteins on 2D gels, has been described (11, 12). The sample/22% interface was enriched in ER, the 22/30% interface was enriched in tonoplast and contained some Golgi membranes, and the 34/40% interface was enriched in PM.

In some experiments, roots of different developmental ages were prepared from control plants. Root tips are defined here as the apical 2 cm of the roots and matured root tissue as that portion of the roots 2 cm or more from the root apex.

The protein in the fractions was assayed by the method of Lowry *et al.* (23) after precipitation with TCA.

Lipid Extraction

To inhibit the activity of endogenous lipases, lipids were extracted from the membrane fractions with a mixture of chloroform and isopropanol (22, 33). Isopropanol (2.12 mL) and chloroform (0.6 mL) were mixed with 0.8 mL of membrane fraction to form a monophasic solution. Insoluble proteins were sedimented by centrifugation at 1000*g* for 3 min and the supernatant was drawn off. Chloroform (3.66 mL) and 0.1 M KCl (0.8 mL) were added to the supernatant to produce a biphasic solution. After thorough mixing, the phases were separated by centrifugation and the lower phase was washed 3 times with 1.5 mL aliquots of 0.1 M KCl saturated with chloroform. Proteins which collected at the interface of the two phases were removed with the upper phase and discarded. The lower phase was dried under a stream of N_2 and the lipids were dissolved in 0.5 mL of chloroform. The samples were stored at −20°C until analyzed.

Some samples were extracted with mixtures of chloroform and methanol. For these samples, the membrane fractions were heated to 100°C for 2 min or were left untreated prior to extraction.

Lipid Analyses

Lipids were separated by TLC. TLC plates (Silica gel 60, 20 × 20, 0.25 mm layer thickness, EM Merck) were prerun in chloroform/methanol (2:1) and then activated at 110°C for 60 min. The plates were developed first in chloroform/methanol/ammonium hydroxide/water (65:30:2:2) and air-dried. Then the plate was rotated 90° and developed in chloroform/methanol/acetic acid/water (170:25:25:6). Lipids were located by exposing air-dried plates to I_2 vapors. The most abundant lipids were identified with specific stains for phosphorus, sugar, primary amine, and sterol substituents (22) and by their comigration with standards in the 2D solvent system. For quantitative analysis, phospholipids were scraped from the TLC plates and analyzed by the method of Bartlett (2). Lipids which contained sugar substituents were scraped from the TLC plates, hydrolyzed in 2 N H_2SO_4 at 100°C for 30 min, and assayed for sugars (10). Sterols were scraped from the TLC plates and eluted into chloroform/methanol (2:1). The solvent was separated from the silica gel by centrifugation and evaporated under a stream of N_2. The sterols were quantified by the method of Zlatkis and Zak (35).

Lipid dry weights were determined on 100 μL of the lipid extract in chloroform. The samples were dried at 50°C, stored in a desiccator until cool, then weighed. The procedure was repeated until the samples reached constant weights.

Identification of Sterols

Samples of total membrane lipids were loaded onto silica gel plates (LK6F, Whatman) and developed in chloroform/acetone/acetic acid (50:50:2). Sterols ($R_F = 0.71$) were scraped from the plate and eluted from the silica gel with chloroform/acetone (1:1). The solvent was removed under a stream of N_2 and the samples were dissolved in 20 to 40 μL chloroform. Initially, samples were analyzed and the sterol components were identified by GC-MS (17). For routine quantification, the sterols were analyzed on a HP 5830A gas chromatograph equipped with a flame ionization detector. The sterols were separated on a DB 1701 capillary column (15 m; injection temperature, 250°C; temperature program, 235–275°C for 10 min and held at 275°C for 20 min; carrier gas, He) with cholesterol as an internal standard.

Identification of Glycosylceramides

As a first step in the purification of glycosylceramides, membrane lipids were saponified in 2.0 mL methanolic KOH. After the saponification step, a biphasic Folch solution (14) was prepared by adding 1.5 mL 0.1 M KCl and 4 mL chloroform to the 2 mL of methanolic KOH. The nonsaponified compounds, including the glycosylceramides, partitioned into the lower phase. The upper phase was discarded and the lower

[4] Mention of a specific product name by the U.S. Department of Agriculture does not constitute an endorsement and does not imply a recommendation over other suitable products.

phase was partitioned twice against 1 mL aliquots of fresh upper phase. The lower phase solvent was evaporated under a stream of N_2 and the sample was dissolved in chloroform. To remove the contaminating lipids from the glycosylceramides, the nonsaponifiables were loaded on a Sep-Pak silica cartridge (Waters Assoc.) prewetted with chloroform. Pigments and sterols were eluted with 4 mL chloroform. Steryl glycosides and glycosylceramides were eluted with 4 mL chloroform/methanol (1:1) followed by 4 mL methanol. The methanolic fractions were taken to dryness, suspended in 50 to 100 μL chloroform/methanol (2:1), and spotted onto channeled silica gel TLC plates (LK6DF, Whatman). The plates were developed in chloroform/methanol (100:20). Steryl glycosides had an R_F of 0.63 and glycosylceramides had an R_F of 0.52. Glycosylceramides were scraped from the TLC plates, eluted with chloroform/methanol (2:1), and separated from the silica gel by centrifugation. The solvent volume was reduced under a stream of N_2 and the sample was filtered (0.5 μm Millex-SR, Millipore). After filtration, the remaining solvent was evaporated, and the glycosylceramides were dissolved in 20 to 40 μL chloroform/methanol (2:1) and stored at −20°C.

Mass spectral analyses using ammonia CI-MS and LSIMS were used to establish mol wt for the glycosylceramides. Measurements were made on a VG-70/70 HS mass spectrometer (VG Analytical Ltd, Manchester, England) equipped with a Cs^+ LSIMS ion source (1). For LSIMS, samples were dissolved in a glycerol matrix. Initially, assignment of mol wt from LSIMS spectra was difficult because of the loss of sugar substituents from the compounds. The addition of NaI to a slightly acidic solution of glycerol prevented this loss and the spectra contained prominent sodiated molecular ions (MNa^+). Galactosylceramide (cerebroside) obtained from bovine brain (Sigma) was used as a standard.

RESULTS

Lipid Composition of Membrane Fractions

The membrane fraction collected from each interface of the sucrose step gradient had a characteristic lipid composition and lipid to protein ratio. Data in Table I show that the lipid to protein ratios were different for each membrane fraction and decreased in the order ER>TG>PM as the membranes equilibrated at increasingly lower interfaces in the gradient. The lipid to protein ratios were similar for salt-treated and control plants (Table I). There was a higher yield of total membrane lipids from plants grown in 100 mM NaCl and a proportionately greater recovery of endomembrane lipids as compared to plasma membrane lipids. The salt treatment had no effect on the proportions of total phospholipid, glycolipid, and sterol in the ER and TG fractions. In the PM fraction there was an increase in the proportion of phospholipids, especially with respect to total glycolipids.

Quantitative data for the most abundant lipid classes in each membrane fraction are given in Figure 1. The ER and TG fractions had similar lipid compositions in which phospholipids and glycolipids predominated. Differences occurred mostly in the relative abundance of the lipids within these classes. The ER fraction was most enriched for phospholipids and among these, PC predominated (Fig. 1). It accounted for about 45% of the total lipid on a molar basis. The PC and PE ratio of 3:1 was the highest of any fraction. The ER fraction also contained the highest percentage of PI. Although PC was the most abundant lipid in the TG fraction it accounted for only 35% of the total lipid, and the ratio of PC and PE was about 2:1. The TG fraction had the highest percentage of glycosylceramide.

The PM fraction, in contrast, contained a lower percentage of phospholipid and was greatly enriched in sterols. In the PM fraction, the various sterols, steryl glycosides, and acylated steryl glycosides accounted for about 40% of the total lipid, more than twice the sterol content of the TG fraction and four times the sterol content of the ER fraction. The PM fraction also had the highest percentages of PS and PA. PA accounted for 6% of the total lipid phosphorus, a percentage which made it the fourth most abundant phospholipid constituent of the PM fraction. In the TG and ER fractions the PA content was lower, 2% and 1% of the total lipid phosphorus, respectively. The PC to PE ratio of the PM fraction was only slightly greater than 1:1.

A low PC content and an abundance of PA in lipid extracts can be artifacts of phospholipase D activity during the preparation of membranes and the subsequent lipid extraction of the membrane fraction. This was not likely to be the cause of the higher PA to PC ratio in the PM fraction because several precautions were taken to minimize phospholipase D activity.

Table I. *Effect of 100 mM NaCl on the Lipids in Membrane Fractions Prepared from Barley Roots*
Plants were grown with or without NaCl.

Treatment	Membrane Fraction					
	ER		TG		PM	
	−NaCl	+NaCl	−NaCl	+NaCl	−NaCl	+NaCl
Lipid/protein (mg/mg)	2.47 ± 0.46[a]	2.38 ± 0.43	1.48 ± 0.25	1.48 ± 0.41	1.16 ± 0.16	1.20 ± 0.14
Phospholipid (nmol)[b]	850 ± 150	1050 ± 280	1800 ± 270	2410 ± 300	680 ± 100	780 ± 180
Glycolipid (nmol)[b,c]	190 ± 50	240 ± 40	800 ± 160	1040 ± 180	400 ± 100	340 ± 60
Sterol (nmol)[b]	40 ± 10	70 ± 30	310 ± 40	400 ± 100	490 ± 80	490 ± 120

[a] All values are ± SD. Data are averaged from six replicate experiments. [b] Data for phospholipids, glycolipids, and sterols are expressed as nmol per mL of membrane sample where each mL contains the membranes prepared from approximately 30 g of roots (fresh weight). [c] Glycolipids include data for acylated steryl glycosides, steryl glycosides, glycosylceramides, and galactolipids.

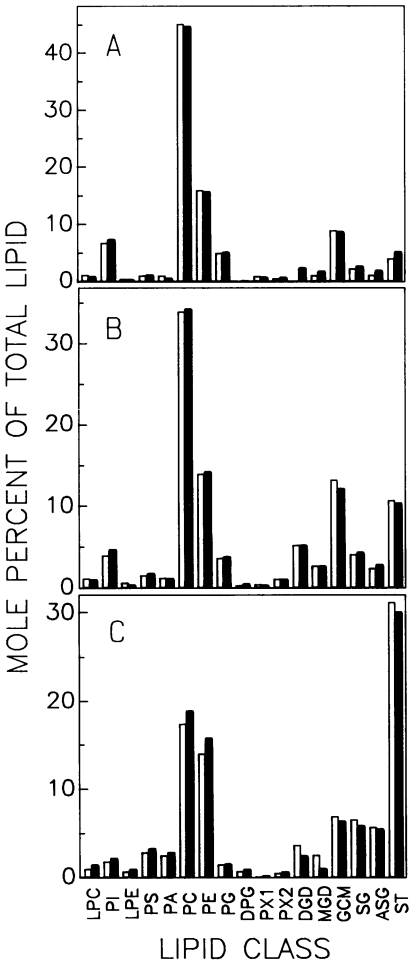

Figure 1. Lipid composition of membrane fractions prepared from barley roots. Barley plants were grown in the presence (solid bar) or absence (open bar) of 100 mM NaCl. A, Composition of the ER fraction; B, composition of the TG fraction; C, composition of the PM fraction. Values are the averages from three replicate experiments with standard errors of approximately 10% of the values shown. Abbreviations: LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; PX1,2, unidentified phospholipids; DGD, digalactosyldiglyceride; MGD, monogalactosyldiglyceride; GCM, glycosylceramide; SG, steryl glycoside; ASG, acylated steryl glycoside; ST, sterol.

The grinding mix contained EDTA and had a high pH, two conditions known to limit phospholipase D as well as many other lipolytic enzymes (15). In addition, preliminary experiments showed that membrane fractions prepared from barley roots contain little phospholipase D activity. When methanol, which stimulates phospholipase D activity (33), was added to a microsomal pellet before the lipids were extracted there was no increase in PA above the levels in a control and there was only a small amount (less than 1% of the total phospholipid) of phosphatidylmethanol, another artifact sometimes produced by phospholipase D activity (data not shown).

Additional phospholipases or lipolytic acyl hydrolases were present in the membrane fractions. Lipolytic activity was observed in experiments when the membrane fractions were extracted in chloroform/methanol (2:1) without prior treatment of the membranes at 100°C (Table II). The chloroform/methanol lipid extracts contained 3 to 5 times more lysoPC, a product of the hydrolysis, than fractions which were extracted in chloroform/isopropanol or which were heated to 100°C before extraction with chloroform/methanol. The greatest content of lysoPC (5% of the total phospholipids) was in the PM fraction. All other data in this paper are from extractions and analyses of heat-treated or chloroform/isopropanol-extracted membrane fractions. Small amounts of lysoPE and lysoPC were still observed on some 2D TLC plates and the lysophospholipids may have been produced during the preparation and fractionation of the membranes despite the precautions which were taken to inhibit lipid-degrading enzymes.

Glycosylceramides

On 2D TLC plates, two spots were identified as glycosylceramides. These lipids were stable to alkaline hydrolysis and contained a sugar substituent but no phosphorus, primary amine, or sterol substituents. LSIMS spectra of the glycolipids contained prominent MNa^+ ions at m/z 864.8 and 866.8. The calculated mol wt for the compounds, 841 and 843, were similar to those of the monoglycosylceramides of PM and tonoplast prepared from plant leaves and hypocotyls (24, 29, 34). The glycosylceramides were the most abundant glycolipid in all three fractions (Fig. 1). The TG fraction contained the highest percentage of glycosylceramides, which accounted for 15% of the total lipid in this fraction. This amount surpassed all phospholipids except PC and approximately equaled the amount of PE and sterol. The glycosylceramides in the ER fraction accounted for about 10% of the total lipid. In the PM fraction glycosylceramides were present in nearly equimolar amounts with the steryl glycosides and the acylated steryl glycosides, each accounting for 7% of the total lipid.

Sterols

Five sterols were observed by GC-MS analysis (Fig. 2). Sitosterol predominated in all three membrane fractions and accounted for about 50% of the sterol in each. The next most abundant sterol was 24ζ-methylcholesterol which accounted for about 40% of the total sterols. The 24ζ-methylcholesterol may be a mixture of campesterol and dihydrobrassicasterol, as has been determined for other members of the Poaceae (17). These sterol epimers vary only in the orientation of the methyl group on carbon 24 and were difficult to separate chromatographically. Stigmasterol was also present in all three membrane fractions and accounted for as little as 5% of the sterol in the ER fraction and up to 12% of the sterol in the PM fraction. Isofucosterol and cycloeucalenol, both intermediates of sitosterol biosynthesis (3, 17), were the only other sterols which were present in large enough quantities to be detected. This is the first report of cylcoeucalenol in barley roots. Both were present in the ER and TG fractions. A trace amount of isofucosterol, but no cycloeucalenol, was present in the PM fraction.

Small differences in sterol composition were observed between control plants and plants grown in 100 mM NaCl (Fig. 2). In each membrane fraction, the percentage of stigmasterol

Table II. *Phospholipid Composition of Membrane Fractions Prepared from Barley Roots*

Membrane fractions were heated to 100°C for 2 min (heat) or remained untreated (no heat) prior to extraction of lipids in a mixture of chloroform and methanol.

Treatment[a]	Membrane Fraction					
	ER		TG		PM	
	Heat	No Heat	Heat	No Heat	Heat	No Heat
	mol %					
LPC	0.7 ± 0.3[b]	2.2 ± 0.8	0.6 ± 0.2	3.2 ± 1.7	0.9 ± 0.2	5.0 ± 2.7
PI + LPE[c]	9.8 ± 2.6	10.0 ± 2.1	7.4 ± 2.0	8.7 ± 2.5	5.1 ± 0.7	7.6 ± 2.7
PS	1.5 ± 0.4	1.4 ± 0.4	2.5 ± 0.6	2.9 ± 0.7	6.6 ± 1.1	7.1 ± 1.5
PA	1.0 ± 0.3	1.0 ± 0.3	1.8 ± 0.5	1.9 ± 0.2	5.6 ± 0.7	6.3 ± 0.8
PC	59.5 ± 13.3	58.0 ± 11.6	57.6 ± 13.1	53.6 ± 12.4	42.7 ± 6.1	36.9 ± 3.0
PE	20.6 ± 4.9	20.9 ± 3.4	23.2 ± 5.1	23.0 ± 4.5	33.8 ± 5.2	32.1 ± 2.1
PG	6.9 ± 1.7	6.4 ± 1.2	6.3 ± 1.5	6.0 ± 1.3	3.6 ± 0.7	3.0 ± 0.3
DPG	0.1 ± 0.1	0.0 ± 0.0	0.6 ± 0.3	0.6 ± 0.3	1.7 ± 0.4	2.0 ± 0.4

[a] LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine. [b] All values are ± SD. Data are averaged from three replicate experiments. [c] PI and LPE were not separated by 2D TLC in all experiments and data for these lipids were combined.

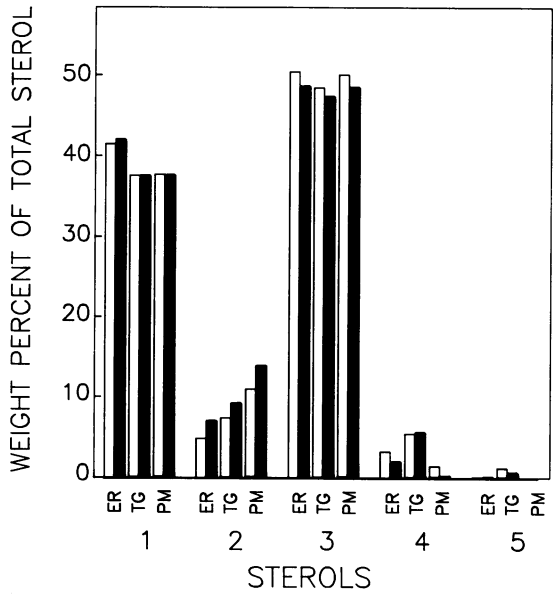

Figure 2. Sterol composition of membrane fractions prepared from barley roots. Barley plants were grown in the presence (solid bar) or absence (open bar) of 100 mM NaCl. Values are the averages from three replicate experiments with standard errors less than 5% of the values shown. 1, 24ζ-Methyl cholesterol; 2, stigmasterol; 3, sitosterol; 4, isofucosterol; 5, cycloeucalenol.

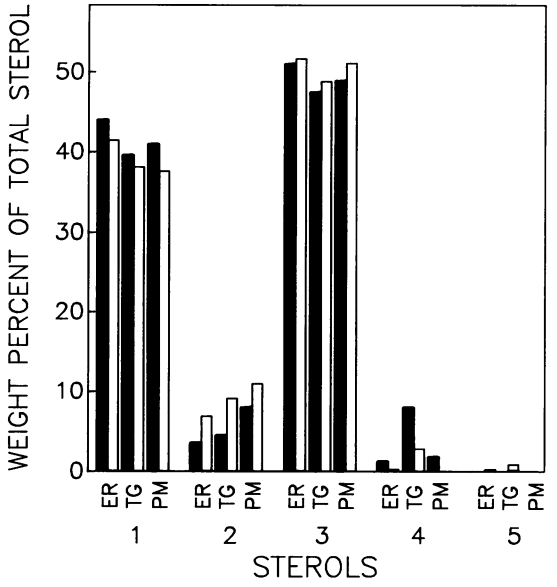

Figure 3. Sterol composition of membrane fractions prepared from barley roots of differing age. Barley roots were divided into root tips (solid bar), the apical 2 cm of the roots, and matured root tissue (open bar), that portion of the roots 2 cm or more from the root apex. Values are the averages from duplicate experiments. 1, 24-ζ-Methyl cholesterol; 2, stigmasterol; 3, sitosterol; 4, isofucosterol; 5, cycloeucalenol.

was greater in salt-treated plants than in controls. The increase in the percentage of stigmasterol for the PM fraction was typical. The percentage of stigmasterol was 11% for the controls and 14% for the plants grown in 100 mM NaCl. There also was a small decrease in sitosterol in salt-treated plants. No change in the percentage of 24ζ-methylcholesterol was detected.

When barley plants were grown in full nutrients and their roots were apportioned into root tips and matured root tissue, unique sterol compositions were found for the two tissues (Fig. 3). There were higher percentages of both sitosterol and stigmasterol in matured root tissue than in root tips. As a percent of total sterols, stigmasterol content was three to five percentage points higher in matured root tissue than in root tips and sitosterol content was one to two percentage points higher. The percentages of 24ζ-methylcholesterol and isofucosterol were highest in root tips. Cycloeucalenol was detected only in matured root tissue.

DISCUSSION

The plasma membrane and the endomembranes had distinct lipid compositions, which were maintained despite ex-

posing the roots to 100 mM NaCl. The compositions were consistent with those reported for PM (30, 34) and endomembranes (25–27, 34) prepared independently from a variety of plant species and tissues. The use of a single source material suggests that the differences between the membranes are under cellular control and not solely caused by species differences in lipid biosynthesis and composition. The differences may reflect the special functions of each membrane, both its physical requirements as a barrier between cellular compartments and its biochemical functions.

For example, the high percentage of glycosylceramide in the TG fraction is consistent with the Golgi membrane as the putative site of ceramide glycosylation and the tonoplast and lysosomal membranes as the cellular destinations of these lipids (31). Glycosylceramides are also abundant in the myelin membrane of neural axons and the brush border membranes of the intestine and the kidney where they are believed to stabilize the membranes and reduce their permeability to ions (6). They may be important in tonoplast and plasma membranes of plants for the same reasons since large ion gradients are maintained across these membranes. Glycosylceramides are present in tonoplast and PM preparations from leaves and hypocotyls (24, 29, 34), and they appear to be the second most abundant glycolipids in plant cells after the galactolipids which are present in plastid membranes.

The 6% PA found in PM prepared from barley roots is high when compared to the amounts of PA found in whole tissue extracts, usually 1 to 2%, but is in the lower range of values previously reported for PM preparations. In PM preparations, concentrations of PA ranging from 5 to 25% of the total lipid phosphorus have been reported. The highest percentage was reported for the Pm prepared from the inflorescence of cauliflower (30), a tissue which is known to be very high in phospholipase D activity (28). In that case it was not clear if phospholipase D was inactivated properly during lipid extraction. High percentages of PA have also been observed when inhibitors of phospholipase D have been added during membrane preparation and lipid extraction procedures have been used which should reduce or eliminate phospholipase D activity. A PA content of 16% of total lipid phosphorus was reported for PM from mung bean hypocotyls in spite of extensive precautions to inactivate phospholipase D (34).

Small changes in the relative abundance of the various sterols were the only differences in lipid composition observed when membranes were prepared from the roots of plants grown in 100 mM NaCl. Stigmasterol content was higher and sitosterol content was lower in all three fractions. A study of the sterol composition of different *Citrus* genotypes of varying salt tolerance also indicated that the relative abundance of the sterols was altered by salt treatment (8). For most *Citrus* cultivars there was a positive correlation between the concentration of NaCl in the soil solution and stigmasterol content and a negative correlation between NaCl concentration and campesterol and sitosterol content. As in barley roots the changes were greatest for sitosterol and stigmasterol. While cholesterol is known to affect the permeability of phospholipid bilayers and biological membranes to ions (4) and to reduce the packing distance between phospholipid head groups (7), whether such effects are induced by the sterols commonly found in plants has not been systematically studied. But, based on space-filling models of sterols, Douglas (8) proposed that the ability of the various plant sterols to reduce permeability may differ. He proposed a model which related sterol structure, membrane sterol composition, and membrane permeability to the ability of *Citrus* seedlings to exclude Cl- and tolerate salt. The model predicts that membranes with higher levels of campesterol would be less permeable to ions and would provide an advantage to plants growing in saline environments. Likewise, a reduction in the percentage of campesterol would be detrimental when salt concentration increased. For the *Citrus* cultivars, regardless of the level of salt tolerance, there was a small decrease in the percentage of campesterol in response to salt treatment. In the barley roots there was no effect of salt on the content of 24ζ-methylcholesterol. Thus, for this relatively salt-tolerant barley cultivar, treatment with 100 mM NaCl did not affect 24ζ-methylcholesterol content even though sitosterol and stigmasterol content were altered.

The changes in sterol composition could be a direct effect of NaCl on lipid metabolism or an indirect effect which resulted from the reduced growth of the roots. It has been reported, for example, that changes in sterol composition accompany the changes in root morphology which occur as roots age (17). The percentages of stigmasterol and sitosterol increased with age of barley root tissue while that of 24ζ-methylcholesterol decreased. The percentage change was greatest for stigmasterol and was similar to the response of both barley and *Citrus* to an increase in salt concentration in the nutrient solution. Thus, for barley, it was not clear whether sterol composition was altered because of the effect of salt on metabolism or on root morphology nor whether the changes in composition were a specific response to NaCl.

Despite the reduction in growth of the barley plants which was induced by 100 mM NaCl, the distinct lipid compositions of the membrane fractions prepared from the roots were maintained. The lipid compositions were unaltered at a salt concentration that induced changes in the proteins associated with the membrane fractions (19, 20) and that induced the activity of a Na^+/H^+ exchanger (16). Thus, while proteins and enzyme activities of each membrane were affected by a salt treatment, the lipid compositions were not. Maintenance of a constant lipid composition that is insensitive to salt may be important for the plant's survival and the different lipid composition of each membrane type is certainly important for the membranes' unique biochemical and physical properties.

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